(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
4 September 2003 (04.09.2003)

PCT

(10) International Publication Number
WO 03/072090 A2

(51) International Patent Classification[7]: A61K 31/00

(21) International Application Number: PCT/IB03/01425

(22) International Filing Date: 26 February 2003 (26.02.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/359,652 27 February 2002 (27.02.2002) US

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)*
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for all designations*

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/072090 A2

(54) Title: USE OF TYROSINE KINASE INHIBITORS FOR TREATING CNS DISORDERS

(57) Abstract: The present invention relates to a method for treating CNS disorders, more particularly selected from the group consisting of depression, schizophrenia, anxiety, migraine, memory loss, pain and neurodegenerative diseases, comprising administering a compound capable of depleting mast cells to a human in need of such treatment. Such compounds can be chosen from tyrosine kinase inhibitors and more particularly non-toxic, selective and potent c-kit inhibitors. Preferably, said inhibitor is unable to promote death of IL-3 dependent cells cultured in presence of IL-3.

Use of tyrosine kinase inhibitors for treating CNS disorders

The present invention relates to a method for treating CNS disorders, more particularly selected from the group consisting of depression, schizophrenia, anxiety, migraine, memory loss, pain and neurodegenerative diseases, comprising administering a compound capable of depleting mast cells to a human in need of such treatment. Such compounds can be chosen from tyrosine kinase inhibitors and more particularly non-toxic, selective and potent c-kit inhibitors. Preferably, said inhibitor is unable to promote death of IL-3 dependent cells cultured in presence of IL-3.

Neurons propagate a signal in the form of an action potential along its axon to other neurons or to effector cells. Many positive or negative signals are exchanged between neurons and are integrated to produce meaningful firing patterns. The communication between two neurons is based on the action of numerous neurotransmitters on specific receptors located at the synapses. A disruption in the regulation of neurotransmission is responsible for neurologic and psychiatric diseases. Furthermore, the activity of neurotransmitters on their respective receptor is normally time limited so that receptors can respond repeatedly to the next waves of stimuli. In this regard, different mechanisms abolish the action of neurotransmitters : they can be pumped back into the presynaptic nerve terminals by active processes (reuptake), they can be destroyed by enzymes, or they simply diffuse into the surrounding area.

According to The Merck Manual of Diagnosis and Therapy, Section 14, Neurologic Disorders, changes in neurotransmitter synthesis, storage, release, or degradation or changes in the number and affinity of receptors can affect neurotransmission and cause clinical disorders.

Among neurotransmitters, we can cite glutamate and aspartate, which are the major excitatory neurotransmitters, whereas aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the brain. But, the first theory about depression concerned the noradrenergic system (NS) (Shildkraut J. et al. 1965, Am J. Psychiat. 122, 509-522). At that time, it was observed that tricyclic compounds (ADT) and monoamine-oxidase inhibitors modified the level of noradrenaline. Later on, in 1978, Sulser F. et al., Biochem Pharmacol. 27, 257-261 showed that these antidepressants lead to a decrease in the number of post-synaptic β-adrenergic receptors. Therefore, it was thought that depression was due to the deregulation of the noradrenergic pre-synaptic stimuli as well as the post-synaptic receptors (Siever LJ. et al. (1985), Am. J. Psychiat. 142, 1017-1031). In 1986, Rasmussen et al., Brain Res. 385, 395-400 demonstrated the presence of serotonin (5-hydroxytriptamin, 5-HT) receptors in NA neurones. Treatments with ADT was shown to provoke also a down-regulation of the 5-HT2 receptors in Sugrue M.F. et al, 1981, Pharmacol. Ther. 13, 219-247. As a consequence, it appears that the NA and 5-HT systems play a crucial role in the regulation of mood and behaviour.

In the nineties, research has focused on the finding of specific serotonin re-uptake inhibitors (SSRI), such as fluoxetin, parxetin or sertralin (Pinder R.M. et al., 1993, Med. Res. Rev. 13, 259-325). Serotonin (5-hydroxytryptamine, or 5-HT) levels are controlled by the uptake of tryptophan and intraneuronal monoamine oxidase activity.

In the meantime, a decrease in the level of HVA, the main catabolic of dopamin (DA), was observed in depressed patients (Kapur S. et al., 1992, Biol. Psychiat. 32, 1-17).
GABA was also shown to be involved in the physiopathology of depression since (i) unipolar patients display decreased level of GABA, (ii) some antidepressants induce the

release of GABA in vivo and (iii) agonists of GABA receptors have antidepressant effects (Lloyd K.G. et al., 1989, Prog. Neuro-Psychopharmacol. Biol. Psychiat. 13, 341-351).

More recently, it has been reported that other factors may be involved in CNS disorders. For example, it has been observed from 30 to 70 % of patients afflicted with melancholia have high level of plasmatic cortisol and escape to the test with dexamethasone described in Caroll B.J. et al. (1981), Arch. Gen. Psychiat. 38, 15.
In addition, corticosteroïds modify (i) the expression of serotoninergic receptors and (ii) the activity of tryptophan hydroxylase, which is the key enzyme in the synthesis of 5-HT (Biegon A., 1990, Ann. NY Acad. Sci. 600, 427-431).

Regarding post-partum or post-menopause depression, repeated administration of oestrogene induces a down-regulation of dopaminergic D2 receptors (Munemura M. et al., 1989, Endocrinology 124, 346-355 and Roy E.J. et al., 1990, Brain. Res. Bull. 25, 221-227).

Other neurotransmitters include the well known acetylcholine, norepinephrine which interacts with adrenergic receptors and which is regulated by tyrosine hydroxylase and monoamine oxidase, endorphins which are polypeptides that activate many central neurons and interact with opioid receptors, enkephalins, dynorphins, histamine, vasopressin, vasoactive intestinal peptide, carnosine, bradykinin, cholecystokinin, bombesin, somatostatin, corticotropin releasing factor, neurotensin, and adenosine.

As mentioned above, any imbalance in these neurotransmitters or any deregulation of associated receptors may lead to the development of CNS disorders ranging from psychiatric diseases to migraine, pain, memory loss and nerve cells degeneracy.

As of today, available treatments include selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine, sertraline, paroxetine, and fluvoxamine. Other compounds include nefazodone which blocks the 5-HT2 receptor and inhibits reuptake of 5-HT and norepinephrine, trazodone which is a 5-HT2 receptor blocker and a 1-noradrenergic blocker, mirtazapine which blocks 2-adrenergic autoreceptors as well as 5-HT2, 5-HT3 and H1 receptors, tricyclic compounds such as imipramine and desipramine, tetracyclic compounds which increase the level of free norepinephrine and of 5-HT, and monoamine oxidase inhibitors (MAOI) which inhibit the oxidative deamination of norepinephrine, dopamine, and 5-HT. We can also cite lithium-antidepressants for treating bipolar disorder.

However, these compounds are only effective in about 65% of depressed patients, which implies a large population afflicted with the so-called "refractory depression". In some cases, the life of patients is in jeopardy at the extent that hospitalization and electroconvulsive therapy is required. This shows the seriousness of these diseases. Furthermore, the above mentioned compounds display numerous side effect such as tachycardia, sedation and weight gain.

Schizophrenia is also a serious mental disorder affecting about 1% of western countries population. Antipsychotic (neuroleptic) drugs available include chlorpromazine and haloperidol which show affinity for the dopamine 2 receptor. But, adverse side effects such as sedation, dystonia, tremors and akathisia have been commonly observed and a significant percentage of patients do not respond to the treatments.

Therefore, the problem is to find alternative solutions to provide a relief and a cure for the numerous patients afflicted with these diseases.

In connection with the present invention, we propose that mast cells are involved in or contribute to CNS disorders. Mast cells (MC) are tissue elements derived from a particular subset of hematopoietic stem cells that express CD34, c-kit and CD13 antigens (Kirshenbaum et al, Blood. 94: 2333-2342, 1999 and Ishizaka et al, Curr Opin Immunol. 5: 937-43, 1993). Immature MC progenitors circulate in the bloodstream and differentiate in tissues. These differentiation and proliferation processes are under the influence of cytokines, one of utmost importance being Stem Cell Factor (SCF), also termed Kit ligand (KL), Steel factor (SL) or Mast Cell Growth Factor (MCGF). SCF receptor is encoded by the protooncogene c-kit, that belongs to type III receptor tyrosine kinase subfamily (Boissan and Arock, J Leukoc Biol. 67: 135-48, 2000). This receptor is also expressed on others hematopoietic or non hematopoietic cells. Ligation of c-kit receptor by SCF induces its dimerization followed by its transphosphorylation, leading to the recruitement and activation of various intracytoplasmic substrates. These activated substrates induce multiple intracellular signaling pathways responsible for cell proliferation and activation (Boissan and Arock, 2000). Mast cells are characterized by their heterogeneity, not only regarding tissue location and structure but also at the functional and histochemical levels (Aldenborg and Enerback., Histochem. J. 26: 587-96, 1994 ; Bradding et al. J Immunol. 155: 297-307, 1995 ; Irani et al, J Immunol. 147: 247-53, 1991 ; Miller et al, Curr Opin Immunol. 1: 637-42, 1989 and Welle et al, J Leukoc Biol. 61: 233-45, 1997).

Here, it is postulated that the activation of mast cells by different stimuli such as stress, trauma, infection as well as neurotransmitters, participate in the exacerbation of the chemical imbalance causing CNS disorders.

More specifically, mast cell degranulation is stimulated by common neurotransmitters such as neurotensin, somatostatin, substance P and acetylcholine, by growth or survival factors, notably NGF, TGFβ1 (see figure 1). Mast cells involved in the response to such stimulus can be brain mast cells but also other mast cells releasing the content of their granules in the blood stream that ultimately reach sensory, motor or brain neurons. Brain mast cells staining is CTMC staining-like but they show the secretory pattern of MMC, implying that they constitute a particular subset of mast cells presenting specificities.

Following mast cells activation, released granules liberate various factors capable of modulating and altering neurotransmission and neurons survival. Among such factors, serotonin is important since an increase of the level of free serotonin has been observed in depressed patients. Alternatively, the sudden burst of serotonin may be followed by a period of serotonin shortage, leading to pain and migraine. As a consequence, we believe that mast cells exacerbate in autocrine or paracrine manner the deregulation of neurotransmission. For example, anxiety or stress-induced release of neurotransmitters such as serotonin activates mast cells, which in turn release the content of their granules, further contributing to the chemical imbalance in the brain leading to CNS disorders. Other mediators released by mast cells can be categorized into vasoactive, nociceptive, proinflammatory and other neurotransmitters. Taken together, these factors are able to induce great disturbance in the activity of neurons, whether they are sensory, motor, or CNS neurons.

We also observed that patients afflicted with mastocytosis are more incline to develop CNS disorders than the normal population. This can be explained by the presence of activating mutations in the c-kit receptor, which induce degranulation of mast cells and a burst of factors contributing to chemical imbalance and neurotransmission alteration.

In some cases, activated mast cells can also participate in the destruction of neuronal tissues by releasing a cocktail of different proteases and mediators categorized into three groups: preformed granule-associated mediators (histamine, proteoglycans, and neutral proteases), lipid-derived mediators (prostaglandins, thromboxanes and leucotrienes), and various cytokines (IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, TNF-α, GM-CSF, MIP-1a, MIP-1b, MIP-2 and IFN-γ). Then, liberation by activated mast cells of mediators (TNF-α, histamine, leucotrienes, prostaglandines etc...) as well as proteases is proposed here i) to induce inflammation and vasodilatation and ii) to participate in the neuronal tissue destruction process.

As a consequence, the present invention proposes to deplete mast cells using compounds that are substantially specific to mast cells. In this regard, tyrosine kinase inhibitors and more particularly c-kit specific kinase inhibitors are proposed to inhibit mast cell proliferation, survival and activation.

A new route for treating CNS disorders is provided, which consists of destroying mast cells involved in and contributing to the pathogenesis of these disorders. It has been found that tyrosine kinase inhibitors and more particularly c-kit inhibitors are especially suited to reach this goal.

Description

The present invention relates to a method for treating CNS disorders comprising administering a compound capable of depleting mast cells to a human in need of such treatment.

Said method for treating CNS disorders can comprise administering a tyrosine kinase inhibitor to a human in need of such treatment.

Tyrosine kinase inhibitors are selected for example from bis monocyclic, bicyclic or heterocyclic aryl compounds (WO 92/20642), vinylene-azaindole derivatives (WO 94/14808) and 1-cycloproppyl-4-pyridyl-quinolones (US 5,330,992), Styryl compounds (US 5,217,999), styryl-substituted pyridyl compounds (US 5,302,606), seleoindoles and selenides (WO 94/03427), tricyclic polyhydroxylic compounds (WO 92/21660) and benzylphosphonic acid compounds (WO 91/15495), pyrimidine derivatives (US 5,521,184 and WO 99/03854), indolinone derivatives and pyrrol-substituted indolinones (US 5,792,783, EP 934 931, US 5,834,504, US 5,883,116, US 5,883,113, US 5, 886,020, WO 96/40116 and WO 00/38519), as well as bis monocyclic, bicyclic aryl and heteroaryl compounds (EP 584 222, US 5,656,643 and WO 92/20642), quinazoline derivatives (EP 602 851, EP 520 722, US 3,772,295 and US 4,343,940) and aryl and heteroaryl quinazoline (US 5,721,237, US 5,714,493, US 5,710,158 and WO 95/15758).

Preferably, said tyrosine kinase inhibitors are unable to promote death of IL-3 dependent cells cultured in presence of IL-3.

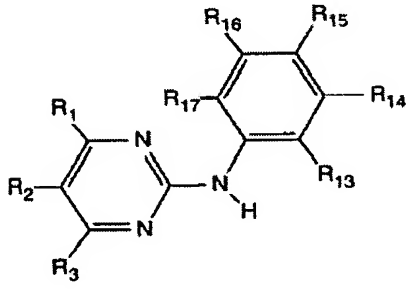
In another embodiment, the invention is directed to a method for treating CNS disorders comprising administering a c-kit inhibitor to a human in need of such treatment.

Preferably, said c-kit inhibitor is a non-toxic, selective and potent c-kit inhibitor. Such inhibitors can be selected from the group consisting of indolinones, pyrimidine derivatives, pyrrolopyrimidine derivatives, quinazoline derivatives, quinoxaline derivatives, pyrazoles derivatives, bis monocyclic, bicyclic or heterocyclic aryl compounds, vinylene-azaindole derivatives and pyridyl-quinolones derivatives, styryl

compounds, styryl-substituted pyridyl compounds, seleoindoles, selenides, tricyclic polyhydroxylic compounds and benzylphosphonic acid compounds.

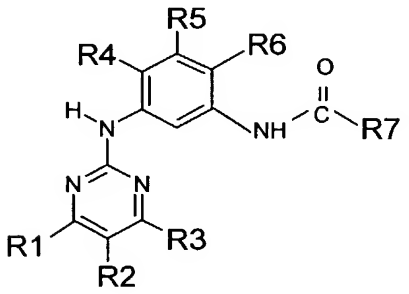
Among preferred compounds, it is of interest to focus on pyrimidine derivatives such as N-phenyl-2-pyrimidine-amine derivatives (US 5,521,184 and WO 99/03854), indolinone derivatives and pyrrol-substituted indolinones (US 5,792,783, EP 934 931, US 5,834,504), US 5,883,116, US 5,883,113, US 5, 886,020, WO 96/40116 and WO 00/38519), as well as bis monocyclic, bicyclic aryl and heteroaryl compounds (EP 584 222, US 5,656,643 and WO 92/20642), quinazoline derivatives (EP 602 851, EP 520 722, US 3,772,295 and US 4,343,940), 4-amino-substituted quinazolines (US 3,470,182), 4-thienyl-2-(1H)-quinazolones, 6,7-dialkoxyquinazolines (US 3,800,039), aryl and heteroaryl quinazoline (US 5,721,237, US 5,714,493, US 5,710,158 and WO 95/15758), 4-anilinoquinazoline compounds (US 4,464,375), and 4-thienyl-2-(1H)-quinazolones (US 3,551,427).

So, preferably, the invention relates to a method for treating CNS disorders comprising administering a non toxic, potent and selective c-kit inhibitor is a pyrimidine derivatives, more particularly N-phenyl-2-pyrimidine-amine derivatives of formula I :



wherein the R1, R2, R3, R13 to R17 groups have the meanings depicted in EP 564 409 B1, incorporated herein in the description.

Preferably, the N-phenyl-2-pyrimidine-amine derivative is selected from the compounds corresponding to formula II :

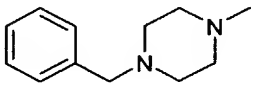


Wherein R1, R2 and R3 are independently chosen from H, F, Cl, Br, I, a C1-C5 alkyl or a cyclic or heterocyclic group, especially a pyridyl group;

R4, R5 and R6 are independently chosen from H, F, Cl, Br, I, a C1-C5 alkyl, especially a methyl group;

and R7 is a phenyl group bearing at least one substituent, which in turn possesses at least one basic site, such as an amino function.

Preferably, R7 is the following group :



Among these compounds, the preferred are defined as follows :

R1 is a heterocyclic group, especially a pyridyl group,

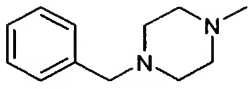
R2 and R3 are H,

R4 is a C1-C3 alkyl, especially a methyl group,

R5 and R6 are H,

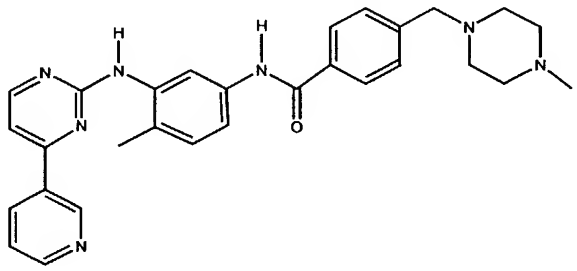
and R7 is a phenyl group bearing at least one substituent, which in turn possesses at least one

basic site, such as an amino function, for example the group :



Therefore, in a preferred embodiment, the invention relates to a method for treating CNS disorders comprising the administration of an effective amount of the compound known in the art as CGP57148B :

4-(4-méhylpipérazine-1-ylméthyl)-N-[4-méthyl-3-(4-pyridine-3-yl)pyrimidine-2 ylamino)phényl]-benzamide corresponding to the following formula :



The preparation of this compound is described in example 21 of EP 564 409 and the β-form, which is particularly useful is described in WO 99/03854.

Alternatively, the c-kit inhibitor can be selected from :

- indolinone derivatives, more particularly pyrrol-substituted indolinones,
- monocyclic, bicyclic aryl and heteroaryl compounds, quinazoline derivatives,
- and quinaxolines, such as 2-phényl-quinaxoline derivatives, for example 2-phenyl-6,7-dimethoxy quinaxoline.

In a preferred aspect, the invention contemplated the method mentioned above, wherein said c-kit inhibitor is unable to promote death of IL-3 dependent cells cultured in presence of IL-3.

The CNS disorders as referred herein include but are not limited to psychiatric disorders, migraine, pain, memory loss and nerve cells degeneracy.

More particularly, the method according to the invention is useful for the treatment of the following disorders :

- Depression including dysthymic disorder, cyclothymic disorder, bipolar depression, severe or "melancholic" depression, atypical depression, refractory depression, seasonal depression, anorexia, bulimia, premenstrual syndrome, post-menopause syndrome.
- Other syndromes such as mental slowing and loss of concentration, pessimistic worry, agitation, self-deprecation, decreased libido,
- Pain including, acute pain, postoperative pain, chronic pain, nociceptive pain, cancer pain, neuropathic pain, psychogenic pain syndromes,
- Anxiety disorders including anxiety associated with hyperventilation and cardiac arrhythmias, phobic disorders, obsessive-compulsive disorder, posttraumatic stress disorder, acute stress disorder, generalized anxiety disorder,
- Psychiatric emergencies such as panic attacks, including psychosis, delusional disorders, conversion disorders, phobias, mania, delirium, dissociative episodes including dissociative amnesia, dissociative fugue and dissociative identity disorder, depersonalization, catatonia, seizures
- Severe psychiatric emergencies including suicidal behaviour, self-neglect, violent or aggressive behaviour, trauma, borderline personality, and acute psychosis,
- Schizophrenia including paranoid schizophrenia, disorganized schizophrenia, catatonic schizophrenia, and undifferentiated schizophrenia,
- Neurodegenerative diseases including Alzheimer's disease , Parkinson's disease, Huntington's disease, the prion diseases, Motor Neurone Disease (MND), and Amyotrophic Lateral Sclerosis (ALS).

Therefore, in a preferred embodiment, the method of the invention is applicable to the treatment of depression.

In another preferred embodiment, the method of the invention is applicable to the treatment of pain.

In another preferred embodiment, the method of the invention is applicable to the treatment of anxiety disorders.

In another preferred embodiment, the method of the invention is applicable to the treatment of psychiatric disorders.

In another preferred embodiment, the method of the invention is applicable to the treatment of schizophrenia.

In another preferred embodiment, the method of the invention is applicable to the treatment of neurodegenerative diseases.

The method as depicted above is also useful for treating memory loss.

In still another preferred embodiment, the method of the invention is applicable to the treatment of migraine.

In a further embodiment, c-kit inhibitors as mentioned above are inhibitors of activated c-kit. In frame with the invention, the expression "activated c-kit" means a constitutively activated-mutant c-kit including at least one mutation selected from point mutations, deletions, insertions, but also modifications and alterations of the natural c-kit sequence (SEQ ID N°1). Such mutations, deletions, insertions, modifications and alterations can occur in the transphosphorylase domain, in the juxtamembrane domain as well as in any domain directly or indirectly responsible for c-kit activity. The expression "activated c-kit" also means herein SCF-activated c-kit. Preferred and optimal SCF concentrations for activating c-kit are comprised between 5.10^{-7} M and 5.10^{-6} M, preferably around

2.10^{-6} M. In a preferred embodiment, the activated-mutant c-kit in step a) has at least one mutation proximal to Y823, more particularly between amino acids 800 to 850 of SEQ ID No1 involved in c-kit autophosphorylation, notably the D816V, D816Y, D816F and D820G mutants. In another preferred embodiment, the activated-mutant c-kit in step a) has a deletion in the juxtamembrane domain of c-kit. Such a deletion is for example between codon 573 and 579 called c-kit d(573-579). The point mutation V559G proximal to the juxtamembrane domain c-kit is also of interest.

In this regard, the invention contemplates a method for treating CNS disorders as defined above comprising administering to a human in need of such treatment a compound that is a selective, potent and non toxic inhibitor of activated c-kit obtainable by a screening method which comprises :

a) bringing into contact (i) activated c-kit and (ii) at least one compound to be tested; under conditions allowing the components (i) and (ii) to form a complex,

b) selecting compounds that inhibit activated c-kit,

c) testing and selecting a subset of compounds identified in step b), which are unable to promote death of IL-3 dependent cells cultured in presence of IL-3.

This screening method can further comprise the step consisting of testing and selecting a subset of compounds identified in step b) that are inhibitors of mutant activated c-kit (for example in the transphosphorylase domain), which are also capable of inhibiting SCF-activated c-kit wild.

Alternatively, in step a) activated c-kit is SCF-activated c-kit wild.

A best mode for practicing this method consists of testing putative inhibitors at a concentration above 10 μM in step a). Relevant concentrations are for example 10, 15, 20, 25, 30, 35 or 40 μM.

In step c), IL-3 is preferably present in the culture media of IL-3 dependent cells at a concentration comprised between 0.5 and 10 ng/ml, preferably between 1 to 5 ng/ml.

Examples of IL-3 dependent cells include but are not limited to :

- cell lines naturally expressing and depending on c-kit for growth and survival. Among such cells, human mast cell lines can be established using the following procedures : normal human mast cells can be infected by retroviral vectors containing sequences coding for a mutant c-kit comprising the c-kit signal peptide and a TAG sequence allowing to differentiate mutant c-kits from c-kit wild expressed in hematopoetic cells by means of antibodies.

This technique is advantageous because it does not induce cellular mortality and the genetic transfer is stable and gives satisfactory yields (around 20 %). Pure normal human mast cells can be routinely obtained by culturing precursor cells originating from blood obtained from human umbilical vein. In this regard, heparinated blood from umbilical vein is centrifuged on a Ficoll gradient so as to isolate mononucleated cells from other blood components. CD34+ precursor cells are then purified from the isolated cells mentioned above using the immunomagnetic selection system MACS (Miltenyi biotech). CD34+ cells are then cultured at 37°C in 5 % CO_2 atmosphere at a concentration of 10^5 cells per ml in the medium MCCM (α-MEM supplemented with L-glutamine, penicillin, streptomycin, 5 10^{-5} M β-mercaptoethanol, 20 % veal fœtal serum, 1 % bovine albumin serum and 100 ng/ml recombinant human SCF. The medium is changed every 5 to 7 days. The percentage of mast cells present in the culture is assessed each week, using May-Grünwal Giemsa or Toluidine blue coloration. Anti-tryptase antibodies can also be used to detect mast cells in culture. After 10 weeks of culture, a pure cellular population of mast cells (> 98 %) is obtained.

It is possible using standard procedures to prepare vectors expressing c-kit for transfecting the cell lines established as mentioned above. The cDNA of human c-kit has been described in Yarden et al., (1987) EMBO J.6 (11), 3341-3351. The coding part of c-kit (3000 bp) can be amplified by PCR and cloned, using the following oligonucleotides :

- 5'AAGAAGAGATGGTACCTCGAGGGGTGACCC3' (SEQ ID No2) sens
- 5'CTGCTTCGCGGCCGCGTTAACTCTTCTCAACCA3' (SEQ ID No3) antisens

The PCR products, digested with Not1 and Xho1, has been inserted using T4 ligase in the pFlag-CMV vector (SIGMA), which vector is digested with Not1 and Xho1 and dephosphorylated using CIP (Biolabs). The pFlag-CMV-c-kit is used to transform bacterial clone XL1-blue. The transformation of clones is verified using the following primers :

- 5'AGCTCGTTTAGTGAACCGTC3' (SEQ ID No4) sens,
- 5'GTCAGACAAAATGATGCAAC3' (SEQ ID No5) antisens.

Directed mutagenesis is performed using relevant cassettes is performed with routine and common procedure known in the art..

The vector Migr-1 (ABC) can be used as a basis for constructing retroviral vectors used for transfecting mature mast cells. This vector is advantageous because it contains the sequence coding for GFP at the 3' and of an IRES. These features allow to select cells infected by the retrovirus using direct analysis with a fluorocytometer. As mentioned above, the N-terminal sequence of c-kit c-DNA can be modified so as to introduce a Flag sequence that will be useful to discriminating heterogeneous from endogenous c-kit.

Other IL-3 dependent cell lines that can be used include but are not limited to:

- BaF3 mouse cells expressing wild-type or mutated form of c-kit (in the juxtamembrane and in the catalytic sites) are described in Kitayama et al, (1996), Blood 88, 995-1004 and Tsujimura et al, (1999), Blood 93, 1319-1329.
- IC-2 mouse cells expressing either c-kit^{WT} or c-kit^{D814Y} are presented in Piao et al, (1996), Proc. Natl. Acad. Sci. USA 93, 14665-14669.

IL-3 independent cell lines are :

- HMC-1, a factor-independent cell line derived from a patient with mast cell leukemia, expresses a juxtamembrane mutant c-kit polypeptide that has constitutive kinase activity (Furitsu T et al, J Clin Invest. 1993;92:1736-1744 ; Butterfield et al, Establishment of an immature mast cell line from a patient with mast cell leukemia. Leuk Res. 1988;12:345-355 and Nagata et al, Proc Natl Acad Sci U S A. 1995;92:10560-10564).
- P815 cell line (mastocytoma naturally expressing c-kit mutation at the 814 position) has been described in Tsujimura et al, (1994), Blood 83, 2619-2626.

The extent to which component (ii) inhibits activated c-kit can be measured *in vitro* or *in vivo*. In case it is measured *in vivo*, cell lines expressing an activated-mutant c-kit, which has at least one mutation proximal to Y823, more particularly between amino acids 800 to 850 of SEQ ID No1 involved in c-kit autophosphorylation, notably the D816V, D816Y, D816F and D820G mutants, are preferred.
Example of cell lines expressing an activated-mutant c-kit are as mentioned above.

In another preferred embodiment, the method further comprises the step consisting of testing and selecting compounds capable of inhibiting c-kit wild at concentration below 1 μM. This can be measured *in vitro* or *in vivo*.

Therefore, compounds are identified and selected according to the method described above are potent, selective and non-toxic c-kit wild inhibitors.

Alternatively, the screening method as defined above can be practiced *in vitro*. In this regard, the inhibition of mutant-activated c-kit and/or c-kit wild can be measured using standard biochemical techniques such as immunoprecipitation and western blot. Preferably, the amount of c-kit phosphorylation is measured.

In a still further embodiment, the invention contemplates a method for treating CNS disorders as depicted above wherein the screening comprises :

a) performing a proliferation assay with cells expressing a mutant c-kit (for example in the transphosphorylase domain), which mutant is a permanent activated c-kit, with a plurality of test compounds to identify a subset of candidate compounds targeting activated c-kit, each having an IC50 < 10 μM, by measuring the extent of cell death,

b) performing a proliferation assay with cells expressing c-kit wild said subset of candidate compounds identified in step (a), said cells being IL-3 dependent cells cultured in presence of IL-3, to identify a subset of candidate compounds targeting specifically c-kit,

c) performing a proliferation assay with cells expressing c-kit, with the subset of compounds identified in step b) and selecting a subset of candidate compounds targeting c-kit wild, each having an IC50 < 10 μM, preferably an IC50 < 1 μM, by measuring the extent of cell death.

Here, the extent of cell death can be measured by 3H thymidine incorporation, the trypan blue exclusion method or flow cytometry with propidium iodide. These are common techniques routinely practiced in the art.

The method according to the invention includes preventing, delaying the onset and/or treating CNS disorders in humans. Regarding the prion diseases, the invention encompasses the treatment of mammals such as bovine and ovine species.

In the method defined above, any compound capable of depleting mast cells can be used. Such compounds can belong to, as explicated above, tyrosine kinase inhibitors, such as c-kit inhibitors, but are not limited to any particular family so long as said compound shows capabilities to deplete mast cells. Depletion of mast cells can be evaluated using for example one of the mast cell lines depicted above using routine procedure.

Best compounds are compounds exhibiting the greatest selectivity.

Control cell lines include other hematopoeitic cells that are not mast cells or related cells or cell lines. These control cell lines include SCF independent expanded human CD34+ normal cells. These control cells also include but are not limited to the human T lymphocyte Jurkat cell line (ATCC N° TIB-152 and mutant cell lines derived thereof), the human B lymphocyte Daudi or Raji cell line (ATCC N° CCL-213 and CCL-86 respectively), the human monocytic U 937 cell line (ATCC N° CRL-1593.2) and the human HL-60 cell line (ATCC N° CCL-240) and mutant cell lines derived thereof CRL-2258 and CRL-2392).

Such compounds can be selected with a method for identifying compounds capable of depleting mast cells, said compound being non-toxic for cell types other than mast cells, comprising the step consisting of :

a) culturing mast cells in vitro in a culture medium suitable for mast cells,

b) adding to said culture medium at least one compound to be tested and incubating said cells for a prolonged period of time,

c) selecting compounds that promote mast cells death,

d) identifying a subset of compounds selected in step c) that are unable to promote death of cells selected from the above mentioned control cell lines.

Therefore, the invention embraces the use of the compounds defined above to manufacture a medicament for treating CNS disorders such as psychiatric disorders, migraine, pain, memory loss and nerve cells degeneracy.

The invention is also directed to the use of the compounds defined above to manufacture a medicament for treating a disorders selected from the subgroups consisting of :

- Depression including dysthymic disorder, cyclothymic disorder, bipolar depression, severe or "melancholic" depression, atypical depression, seasonal depression, anorexia, bulimia, premenstrual syndrome, post-menopause syndrome.
- Other syndromes such as mental slowing and loss of concentration, pessimistic worry, agitation, self-deprecation, decreased libido,
- Pain including, acute pain, postoperative pain, chronic pain, nociceptive pain, cancer pain, neuropathic pain, psychogenic pain syndromes,
- Anxiety disorders including anxiety associated with hyperventilation and cardiac arrhythmias, phobic disorders, obsessive-compulsive disorder, posttraumatic stress disorder, acute stress disorder, generalized anxiety disorder,
- Psychiatric emergencies such as panic attacks, including psychosis, delusional disorders, conversion disorders, phobias, mania, delirium, dissociative episodes including dissociative amnesia, dissociative fugue and dissociative identity disorder, depersonalization, catatonia, seizures
- Severe psychiatric emergencies including suicidal behaviour, self-neglect, violent or aggressive behaviour, trauma, borderline personality, and acute psychosis,
- Schizophrenia including paranoid schizophrenia, disorganized schizophrenia, catatonic schizophrenia, and undifferentiated schizophrenia,

- Neurodegenerative diseases including Alzheimer's disease , Parkinson's disease, Huntington's disease, the prion diseases, Motor Neurone Disease (MND), and Amyotrophic Lateral Sclerosis (ALS).

The pharmaceutical compositions utilized in this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Further details on techniques for formulation and administration may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing Co., Easton, Pa.).

Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

More particularly, the invention relates to a pharmaceutical composition intended for oral administration.

Regarding the treatment of pain, topical administration may be most suitable in some cases. Here, the compositions according to the invention may be presented in the form of a gel, paste, ointment, cream, lotion, liquid suspension aqueous, aqueous-alcoholic or,

oily solutions, or dispersions of the lotion or serum type, or anhydrous or lipophilic gels, or emulsions of liquid or semi-solid consistency of the milk type, obtained by dispersing a fatty phase in an aqueous phase or vice versa, or of suspensions or emulsions of soft, semi-solid consistency of the cream or gel type, or alternatively of microemulsions, of microcapsules, of microparticles or of vesicular dispersions to the ionic and/or nonionic type. These compositions are prepared according to standard methods.

The composition according to the invention comprises any ingredient commonly used in dermatology and cosmetic. It may comprise at least one ingredient selected from hydrophilic or lipophilic gelling agents, hydrophilic or lipophilic active agents, preservatives, emollients, viscosity enhancing polymers, humectants, surfactants, preservatives, antioxidants, solvents, and fillers, antioxidants, solvents, perfumes, fillers, screening agents, bactericides, odor absorbers and coloring matter.

As oils which can be used in the invention, mineral oils (liquid paraffin), vegetable oils (liquid fraction of shea butter, sunflower oil), animal oils, synthetic oils, silicone oils (cyclomethicone) and fluorinated oils may be mentioned. Fatty alcohols, fatty acids (stearic acid) and waxes (paraffin, carnauba, beeswax) may also be used as fatty substances.

As emulsifiers which can be used in the invention, glycerol stearate, polysorbate 60 and the PEG-6/PEG-32/glycol stearate mixture are contemplated.

As hydrophilic gelling agents, carboxyvinyl polymers (carbomer), acrylic copolymers such as acrylate/alkylacrylate copolymers, polyacrylamides, polysaccharides such as hydroxypropylcellulose, clays and natural gums may be mentioned, and as lipophilic gelling agents, modified clays such as bentones, metal salts of fatty acids such as aluminum stearates and hydrophobic silica, or alternatively ethylcellulose and polyethylene may be mentioned.

As hydrophilic active agents, proteins or protein hydrolysates, amino acids, polyols, urea, allantoin, sugars and sugar derivatives, vitamins, starch and plant extracts, in particular those of Aloe vera may be used.

As lipophilic active, agents, retinol (vitamin A) and its derivatives, tocopherol (vitamin E) and its derivatives, essential fatty acids, ceramides and essential oils may be used. These agents add extra moisturizing or skin softening features when utilized.

In addition, a surfactant can be included in the composition so as to provide deeper penetration of the compound capable of depleting mast cells, such as a tyrosine kinase inhibitor, preferably a c-kit inhibitor.

Among the contemplated ingredients, the invention embraces penetration enhancing agents selected for example from the group consisting of mineral oil, water, ethanol, triacetin, glycerin and propylene glycol; cohesion agents selected for example from the group consisting of polyisobutylene, polyvinyl acetate and polyvinyl alcohol, and thickening agents.

Chemical methods of enhancing topical absorption of drugs are well known in the art. For example, compounds with penetration enhancing properties include sodium lauryl sulfate (Dugard, P. H. and Sheuplein, R. J., "Effects of Ionic Surfactants on the Permeability of Human Epidermis: An Electrometric Study," J. Ivest. Dermatol., V.60, pp. 263-69, 1973), lauryl amine oxide (Johnson et. al., US 4,411,893), azone (Rajadhyaksha, US 4,405,616 and 3,989,816) and decylmethyl sulfoxide (Sekura, D. L. and Scala, J., "The Percutaneous Absorption of Alkylmethyl Sulfides," Pharmacology of the Skin, Advances In Biolocy of Skin, (Appleton-Century Craft) V. 12, pp. 257-69, 1972). It has been observed that increasing the polarity of the head group in amphoteric

molecules increases their penetration-enhancing properties but at the expense of increasing their skin irritating properties (Cooper, E. R. and Berner, B., "Interaction of Surfactants with Epidermal Tissues: Physiochemical Aspects," Surfactant Science Series, V. 16, Reiger, M. M. ed. (Marcel Dekker, Inc.) pp. 195-210, 1987).

A second class of chemical enhancers are generally referred to as co-solvents. These materials are absorbed topically relatively easily, and, by a variety of mechanisms, achieve permeation enhancement for some drugs. Ethanol (Gale et. al., U.S. Pat. No. 4,615,699 and Campbell et. al., U.S. Pat. Nos. 4,460,372 and 4,379,454), dimethyl sulfoxide (US 3,740,420 and 3,743,727, and US 4,575,515), and glycerine derivatives (US 4,322,433) are a few examples of compounds which have shown an ability to enhance the absorption of various compounds.

Pharmaceutical compositions suitable for use in the invention include compositions wherein compounds for depleting mast cells, such as tyrosine kinase inhibitors and c-kit inhibitors, are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art. A therapeutically effective dose refers to that amount of active ingredient, which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., ED50 (the dose therapeutically effective in 50% of the population) and LD50 (the dose lethal to 50% of the population). The dose ratio of toxic to therapeutic effects is the therapeutic index, and it can be expressed as the ratio, LD50/ED50. Pharmaceutical compositions which exhibit large therapeutic indices are preferred. As mentioned above, a tyrosine kinase inhibitor and more particularly a c-kit inhibitor according to the invention is unable to promote death of IL-3 dependent cells cultured in presence of IL-3.

The invention also contemplates a product comprising at least one compound capable of depleting mast cells, such as a tyrosine kinase inhibitors, more particularly a non-toxic, selective and potent c-kit inhibitor and at least one antidepressant, antipsychotic, or anxiolytic for simultaneous, separate or sequential use for the treatment of CNS disorders as defined above.

CLAIMS

1. A method for treating CNS disorders comprising administering a compound capable of depleting mast cells to a human in need of such treatment.

2. A method according to claim 1 for treating CNS disorders comprising administering a tyrosine kinase inhibitor to a human in need of such treatment.

3. A method according to claim 2, wherein said tyrosine kinase inhibitor is unable to promote death of IL-3 dependent cells cultured in presence of IL-3.

4. A method according to claim 2 for treating CNS disorders comprising administering a c-kit inhibitor to a human in need of such treatment.

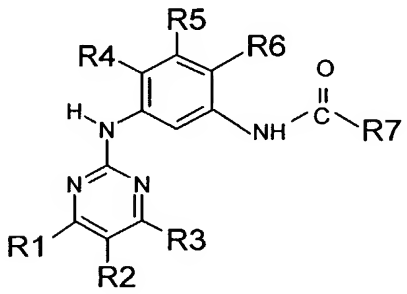
5. A method according to claim 4, wherein said c-kit inhibitor is a non-toxic, selective and potent c-kit inhibitor.

6. A method according to claim 5, wherein said inhibitor is selected from the group consisting of indolinones, pyrimidine derivatives, pyrrolopyrimidine derivatives, quinazoline derivatives, quinoxaline derivatives, pyrazoles derivatives, bis monocyclic, bicyclic or heterocyclic aryl compounds, vinylene-azaindole derivatives and pyridyl-quinolones derivatives, styryl compounds, styryl-substituted pyridyl compounds, seleoindoles, selenides, tricyclic polyhydroxylic compounds and benzylphosphonic acid compounds.

7. A method according to claim 5, wherein said inhibitor is selected from the group consisting of :

- pyrimidine derivatives, more particularly N-phenyl-2-pyrimidine-amine derivatives.
- indolinone derivatives, more particularly pyrrol-substituted indolinones,
- monocyclic, bicyclic aryl and heteroaryl compounds,
- and quinazoline derivatives.

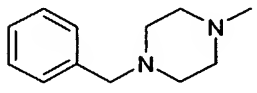
8. A method according to claim 4, wherein said inhibitor is selected from the group consisting of N-phenyl-2-pyrimidine-amine derivatives having the formula II :



Wherein R1, R2 and R3 are independently chosen from H, F, Cl, Br, I, a C1-C5 alkyl or a cyclic or heterocyclic group, especially a pyridyl group;

R4, R5 and R6 are independently chosen from H, F, Cl, Br, I, a C1-C5 alkyl, especially a methyl group;

and R7 is a phenyl group bearing at least one substituent, which in turn possesses at least one basic site, such as an amino function, preferably the following group :



9. A method according to claim 8, wherein said inhibitor is the 4-(4-méhylpipérazine-1-ylméthyl)-N-[4-méthyl-3-(4-pyridine-3-yl)pyrimidine-2 ylamino)phényl]-benzamide.

10. A method according to one of claims 4 to 9, wherein said c-kit inhibitor is unable to promote death of IL-3 dependent cells cultured in presence of IL-3.

11. A method according to one of claims 4 to 10, wherein said c-kit inhibitor is an inhibitor of activated c-kit.

12. A method according to claim 11, wherein said inhibitor is capable of inhibiting constitutively activated-mutant c-kit.

13. A method according to one of claims 4 to 10, wherein said activated c-kit inhibitor is capable of inhibiting SCF-activated c-kit.

14. A method for treating CNS disorders comprising administering to a human in need of such treatment a compound that is a selective, potent and non toxic inhibitor of activated c-kit obtainable by a screening method which comprises :
a) bringing into contact (i) activated c-kit and (ii) at least one compound to be tested; under conditions allowing the components (i) and (ii) to form a complex,
b) selecting compounds that inhibit activated c-kit,
c) testing and selecting a subset of compounds identified in step b), which are unable to promote death of IL-3 dependent cells cultured in presence of IL-3.

15. A method according to claim 14, wherein the screening method further comprises the step consisting of testing and selecting a subset of compounds identified in step b) that are inhibitors of mutant activated c-kit, which are also capable of inhibiting SCF-activated c-kit wild.

16. A method according to claim 14, wherein activated c-kit is SCF-activated c-kit wild in step a).

17. A method according to one of claims 14 to 16, wherein putative inhibitors are tested at a concentration above 10 μM in step a).

18. A method according to one of claims 14 to 16, wherein IL-3 is preferably present in the culture media of IL-3 dependent cells at a concentration comprised between 0.5 and 10 ng/ml, preferably between 1 to 5 ng/ml.

19. A method according to claim 14, wherein IL-3 dependent cells are selected from the group consisting of mast cells, transfected mast cells, BaF3 and IC-2.

20. A method according to one of claims 14 to 19, wherein the extent to which component (ii) inhibits activated c-kit is measured *in vitro* or *in vivo*.

21. A method according to one of claims 14 to 20, further comprising the step consisting of testing and selecting compounds capable of inhibiting c-kit wild at concentration below 1 μM.

22. A method according to claim 17 or 21, wherein the testing is performed *in vitro* or *in vivo*.

23. A method according to one of claims 14 to 22, wherein the inhibition of mutant-activated c-kit and/or c-kit wild is measured using standard biochemical techniques such as immunoprecipitation and western blot.

24. A method according to one of claims 14 to 22, wherein the amount of c-kit phosphorylation is measured.

25. A method according to one of claims 14 to 24, wherein identified and selected compounds are potent, selective and non-toxic c-kit wild inhibitors.

26. A method for treating CNS disorders comprising administering to a human in need of such treatment a c-kit inhibitor obtainable by a screening method comprising :

a) performing a proliferation assay with cells expressing a mutant c-kit (for example in the transphosphorylase domain), which mutant is a permanent activated c-kit, with a plurality of test compounds to identify a subset of candidate compounds targeting activated c-kit, each having an IC50 < 10 μM, by measuring the extent of cell death,

b) performing a proliferation assay with cells expressing c-kit wild said subset of candidate compounds identified in step (a), said cells being IL-3 dependent cells cultured in presence of IL-3, to identify a subset of candidate compounds targeting specifically c-kit,

c) performing a proliferation assay with cells expressing c-kit, with the subset of compounds identified in step b) and selecting a subset of candidate compounds targeting c-kit wild, each having an IC50 < 10 μM, preferably an IC50 < 1 μM, by measuring the extent of cell death.

27. A method according to claim 26, wherein the extent of cell death is measured by 3H thymidine incorporation, the trypan blue exclusion method or flow cytometry with propidium iodide.

28. A method according to one of claims 1 to 27 for preventing, delaying the onset and/or treating CNS disorders in human.

29. A method according to one of claims 1 to 28 for treating psychiatric disorders, migraine, pain, memory loss and nerve cells degeneracy.

30. A method according to one of claims 1 to 28 for treating depression including dysthymic disorder, cyclothymic disorder, bipolar depression, severe or "melancholic" depression, atypical depression, refractory depression, seasonal depression, anorexia, bulimia, premenstrual syndrome and post-menopause syndrome.

31. A method according to one of claims 1 to 28 for treating mental slowing and loss of concentration, pessimistic worry, agitation, self-deprecation and decreased libido.

32. A method according to one of claims 1 to 28 for treating pain including, acute pain, postoperative pain, chronic pain, nociceptive pain, cancer pain, neuropathic pain, and psychogenic pain syndromes.

33. A method according to one of claims 1 to 28 for treating anxiety disorders including anxiety associated with hyperventilation and cardiac arrhythmias, phobic disorders, obsessive-compulsive disorder, posttraumatic stress disorder, acute stress disorder, and generalized anxiety disorder.

34. A method according to one of claims 1 to 28 for treating psychiatric disorders such as panic attacks, including psychosis, delusional disorders, conversion disorders, phobias, mania, delirium, dissociative episodes including dissociative amnesia, dissociative fugue and dissociative identity disorder, depersonalization, catatonia, and seizures.

35. A method according to one of claims 1 to 28 for treating severe psychiatric disorders including suicidal behaviour, self-neglect, violent or aggressive behaviour, trauma, borderline personality, and acute psychosis.

36. A method according to one of claims 1 to 28 for treating schizophrenia including paranoid schizophrenia, disorganized schizophrenia, catatonic schizophrenia, and undifferentiated schizophrenia.

37. A method according to one of claims 1 to 28 for treating neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, Huntington's disease, the prion diseases, Motor Neurone Disease (MND), and Amyotrophic Lateral Sclerosis (ALS).

38. A method according to one of claims 1 to 28 for treating memory loss.

39. A method according to one of claims 1 to 28 for treating migraine.

40. Use of a c-kit inhibitor to manufacture a medicament for treating CNS disorders, more particularly for the treatment of psychiatric disorders, migraine, pain, memory loss and nerve cells degeneracy.

41. A composition suitable for topical administration comprising a compound capable of depleting mast cells, preferably a tyrosine kinase inhibitor, more particularly a c-kit inhibitor for the treatment of pain.

42. A composition suitable for oral administration comprising a compound capable of depleting mast cells, preferably a tyrosine kinase inhibitor, more particularly a c-kit

inhibitor for the treatment of CNS disorders, more particularly for the treatment of psychiatric disorders, migraine, pain, memory loss and nerve cells degeneracy.

43. A composition suitable for intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, enteral, sublingual, or rectal administration comprising a compound capable of depleting mast cells, preferably a tyrosine kinase inhibitor, more particularly a c-kit inhibitor for the treating of CNS disorders, more particularly for the treatment of psychiatric disorders, migraine, pain, memory loss and nerve cells degeneracy.

44. A product comprising at least one compound capable of depleting mast cells, such as a tyrosine kinase inhibitors, more particularly a non-toxic, selective and potent c-kit inhibitor and at least one antidepressant, antipsychotic, or anxiolytic for simultaneous, separate or sequential use for the treatment of CNS disorders, more particularly for the treatment of psychiatric disorders, migraine, pain, memory loss and nerve cells degeneracy.

SEQUENCE LISTING

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<120> Use of tyrosine kinase inhibitors for treating CNS disorders

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<151> 2002-02-27

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Arg Val Gly Asp Glu Ile Arg Leu Leu Cys Thr Asp Pro Gly Phe Val
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145                 150                 155                 160

Arg Phe Ile Pro Asp Pro Lys Ala Gly Ile Met Ile Lys Ser Val Lys
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            260                 265                 270

Ala Thr Leu Thr Ile Ser Ser Ala Arg Val Asn Asp Ser Gly Val Phe
        275                 280                 285

Met Cys Tyr Ala Asn Asn Thr Phe Gly Ser Ala Asn Val Thr Thr Thr
    290                 295                 300

Leu Glu Val Val Asp Lys Gly Phe Ile Asn Ile Phe Pro Met Ile Asn
305                 310                 315                 320

Thr Thr Val Phe Val Asn Asp Gly Glu Asn Val Asp Leu Ile Val Glu
                325                 330                 335

Tyr Glu Ala Phe Pro Lys Pro Glu His Gln Gln Trp Ile Tyr Met Asn
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Thr Glu Gly Gly Thr Tyr Thr Phe Leu Val Ser Asn Ser Asp Val Asn
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Phe Pro Glu Pro Thr Ile Asp Trp Tyr Phe Cys Pro Gly Thr Glu Gln
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Arg Cys Ser Ala Ser Val Leu Pro Val Asp Val Gln Thr Leu Asn Ser
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Tyr Lys Tyr Leu Gln Lys Pro Met Tyr Glu Val Gln Trp Lys Val Val
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Cys Cys Tyr Gly Asp Leu Leu Asn Phe Leu Arg Arg Lys Arg Asp Ser
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Phe Ile Cys Ser Lys Gln Glu Asp His Ala Glu Ala Ala Leu Tyr Lys
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Ile Lys Asn Asp Ser Asn Tyr Val Val Lys Gly Asn Ala Arg Leu Pro
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Val Lys Trp Met Ala Pro Glu Ser Ile Phe Asn Cys Val Tyr Thr Phe
        835                 840                 845

Glu Ser Asp Val Trp Ser Tyr Gly Ile Phe Leu Trp Glu Leu Phe Ser
    850                 855                 860

Leu Gly Ser Ser Pro Tyr Pro Gly Met Pro Val Asp Ser Lys Phe Tyr
865                 870                 875                 880

Lys Met Ile Lys Glu Gly Phe Arg Met Leu Ser Pro Glu His Ala Pro
                885                 890                 895

Ala Glu Met Tyr Asp Ile Met Lys Thr Cys Trp Asp Ala Asp Pro Leu
            900                 905                 910

Lys Arg Pro Thr Phe Lys Gln Ile Val Gln Leu Ile Glu Lys Gln Ile
        915                 920                 925
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Ser Glu Ser Thr Asn His Ile Tyr Ser Asn Leu Ala Asn Cys Ser Pro
930 935 940

Asn Arg Gln Lys Pro Val Val Asp His Ser Val Arg Ile Asn Ser Val
945 950 955 960

Gly Ser Thr Ala Ser Ser Ser Gln Pro Leu Leu Val His Asp Asp Val
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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
4 September 2003 (04.09.2003)

PCT

(10) International Publication Number
WO 03/072090 A3

(51) International Patent Classification[7]: A61K 38/00

(21) International Application Number: PCT/IB03/01425

(22) International Filing Date: 26 February 2003 (26.02.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/359,652 27 February 2002 (27.02.2002) US

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)*
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for all designations*

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report:
13 November 2003

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/072090 A3

(54) Title: USE OF TYROSINE KINASE INHIBITORS FOR TREATING CNS DISORDERS

(57) Abstract: The present invention relates to a method for treating CNS disorders, more particularly selected from the group consisting of depression, schizophrenia, anxiety, migraine, memory loss, pain and neurodegenerative diseases, comprising administering a compound capable of depleting mast cells to a human in need of such treatment. Such compounds can be chosen from tyrosine kinase inhibitors and more particularly non-toxic, selective and potent c-kit inhibitors. Preferably, said inhibitor is unable to promote death of IL-3 dependent cells cultured in presence of IL-3.

INTERNATIONAL SEARCH REPORT

Internati plication No
PCT/IB 03/01425

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K38/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 61028 A (GORBACH SHERWOOD L) 2 December 1999 (1999-12-02) column 3, line 15-21	1,2, 28-44
X	US 5 952 374 A (CLARKSON JR THOMAS BOSTON ET AL) 14 September 1999 (1999-09-14) column 2, line 7-28	1,2,8-44

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the International search report
27 August 2003	02/09/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Cattell, James

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter ... l application No.
PCT/IB 03/01425

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: -
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claim 1relates to compounds defined by reference to a desirable characteristic or property, namely "capable of depleting mast cells"

The claim cover all such compounds, whereas the description is for only a very limited number of such compounds.

An attempt is made to define the compound by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Internati plication No
PCT/IB 03/01425

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9961028 A	02-12-1999	AU 4204099 A	13-12-1999
		CA 2333556 A1	02-12-1999
		EP 1082122 A1	14-03-2001
		WO 9961028 A1	02-12-1999
US 5952374 A	14-09-1999	NONE	